

The Victorian Naturalist

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From the Editors

At this time of year, when the wildflowers are in full bloom and the native gardener is rewarded with the riot of yellows, pinks, blues and whites, one can only wonder at the miracle of nature, and its resilience. As naturalists, we can consider ourselves to be very lucky.

In this issue we have two papers that discuss some of nature's myriad strategies — from alcoholic nectar in *Grevillea* and *Eucalyptus* species to differences in the woody follicles of *Hakea decurrens*.

It is a pity that humankind can't work out its own strategies for sharing with nature. But in his paper on wetland restoration programs (originally presented to the 2013 FNCV Biodiversity Symposium 'Water and Biodiversity') Damien Cook shows us how humankind can work towards repairing the damage it has done to nature's systems.

And the important observations of calling behaviour of the Giant Burrowing Frog in eastern Victoria is discussed by Rohan Bilney.

All of this just makes one think – isn't nature grand?!

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Postal Address: FNCV, PO Box 13, Blackburn, Victoria 3130, Australia.

Phone (03) 9877 9860; International Phone 61 3 9877 9860.

email: admin@fncv.org.au

www.fncv.org.au

Address correspondence to:

The Editors, *The Victorian Naturalist*, PO Box 13, Blackburn, Victoria 3130, Australia.

Phone: (03) 9877 9860. Email: vicnat@fncv.org.au

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Volume 132 (5) 2015

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Editorial Assistant: Virgil Hubregtse

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Front cover: Giant Burrowing Frog *Heleioporus australiacus*. Photo Rohan Bilney. See page 128.
Back cover: Apostlebirds in Mungo National Park. Photo Anne Morton. See page 155.

Observations of Giant Burrowing Frogs *Heleioporus australiacus* (Limnodynastidae) in the Mitchell River catchment, East Gippsland, Victoria

Rohan J Bilney

Forestry Corporation of NSW, PO Box 702, Eden, NSW 2551. Email: rohan.bilney@gmail.com

Abstract

Within Victoria, limited records of the threatened Giant Burrowing Frog have been obtained in recent decades. This paper describes 16 records of calling Giant Burrowing Frogs from four tributaries of the Mitchell River system in the Mount Alfred State Forest and Mitchell River National Park, Victoria. Stream condition was a major determinant of calling activity, with calling detected only in pools with no or slow flow, within first, second and fourth order streams, often with sandstone bases. Calling was not always closely associated with rainfall, and occurred across most seasons. (*The Victorian Naturalist* 132 (5) 2015, 128–133)

Keywords: amphibian, breeding habitat, calling behaviour, *Heleioporus australiacus*

Introduction

The threatened Giant Burrowing Frog *Heleioporus australiacus* (Fig. 1) is a large, cryptic amphibian from south-eastern Australia (Gillespie 1990; Penman *et al.* 2004). Its known range extends from east of Walhalla in Victoria along the Great Dividing Range to Newcastle in NSW, where it has been recorded from various forested habitat types (Gillespie 1990; Penman *et al.* 2004). Despite this wide distribution, limited records exist towards the southern extent of its range in Victoria where it is now considered Critically Endangered compared to a national listing of Vulnerable (DSE 2013). It is so rarely encountered that until recently information on the species' ecology barely extended beyond a limited number of incidental observations (Gillespie 1990; Daly 1996; Penman *et al.* 2004) and a description and sonagram of the call (Littlejohn and Martin 1967). Recent studies on radio-tagged individuals in south-eastern New South Wales investigated important ecological aspects, including spatial ecology, burrowing locations, habitat requirements and meteorological influences on movement (e.g. Lemckert and Brassil 2003; Penman *et al.* 2005a, 2006b, 2008), but there is little information on breeding sites and calling behaviour (Gillespie 1990; Daly 1996; Penman *et al.* 2006c). Anstis (2013) provides an account and illustrations of the life history and larval development of the species in the Sydney area.

The present paper details observations of Giant Burrowing Frogs calling from streams in the Mitchell River catchment in East Gippsland, Victoria.

Methods

Study area

The study area primarily included the Stony Creek catchment (a tributary of the Mitchell River) within the Mount Alfred State Forest and lower Mitchell River National Park, East Gippsland, Victoria. The area is located approximately 220 km east of Melbourne and 20 km north-west of Bairnsdale. Elevation is largely between 50 and 300 m. The vegetation is dominated by Lowland Forest on the ridges and upper slopes, typically with Lowland Herb-rich Forest in the gullies. Dominant overstorey species include *Eucalyptus globoidea*, *E. cypellocarpa*, *E. polyanthemus* and *E. considniana*, and dominant understorey species include *Pomaderris aspera*, *Acacia dealbata*, *A. mearnsii*, *Cassinia* spp., *Gahnia radula*, *Goodenia ovata*, *Kunzea* sp., *Lepidosperma* spp., *Lomandra longifolia*, *Pteridium esculentum* and *Styphandra glauca*.

Surveys

Between 2003 and 2008 two observations of calling Giant Burrowing Frogs were obtained incidentally during nocturnal surveys targeting large forest owls. Between April 2011 and



Fig. 1. A male Giant Burrowing Frog in a typical calling posture and location.

May 2014, 17 periodic targeted surveys for Giant Burrowing Frogs were conducted throughout the Mount Alfred State Forest and Mitchell River National Park, primarily after rainfall (>5 mm) and when it was expected that ephemeral streams would contain water. All surveys were nocturnal and involved either walking along streams listening for calls (usually 200–700 m), or 10 minute listening surveys near roads close to streams. Most surveys were conducted <3 h after dark. When a Giant Burrowing Frog was heard, attempts were usually made to observe the individual and obtain site coordinates using a GPS unit. The site was revisited during daylight hours to measure the water body (maximum width and depth), and record surrounding vegetation. At three sites where calling had been noted, an automated audio recording device (Song Meter SM2+, Wildlife Acoustics, Massachusetts, USA), was deployed in an attempt to record calling behaviour for 3 h after sunset. When repeat visits detected an individual within close proximity to a recent previous detection (within 15 m), it was considered to be

the same individual, even if occupying a different nearby pool or stretch of creek.

Meteorological data were obtained from a weather station at Glenaladale (Site no. 58270) (Bureau of Meteorology) located approximately 5 to 10 km from the study area. Average annual rainfall for the years 2002–2013 was 696 mm (± 127 mm).

Results

Sixteen records of calling Giant Burrowing Frogs were obtained from four separate streams. The probable total number of individual males was nine, with repeat observations of several individuals suspected (Table 1). One female was observed incidentally. It should be noted that searches for egg-masses and tadpoles were not conducted during surveys; however, Giant Burrowing Frog tadpoles were conspicuous throughout the creek at Site B over a nine month period from the initial surveys undertaken in Autumn 2011 until early Summer the same year (searches along the creek itself beyond this date were not undertaken).

Table 1. Calling sites of male Giant Burrowing Frogs.

Site ID	Stream order	Stream/pool width	Max. pool depth (<2 m from frog)	No. of distinct calling sites	No. of individual frogs	Dominant plant species within 30 m
A	4	~10 m	?	?	2	<i>Eucalyptus tereticornis</i> , <i>E. globoidea</i> , <i>Brachychiton populneus</i> , <i>Dodonaea viscosa</i> , <i>Kimzeia</i> sp., <i>Cassinia</i> sp.
B	2	2–3 m	5–40 cm	6	4	<i>E. cypellocarpa</i> , <i>E. globoidea</i> , <i>E. polyanthemos</i> , <i>Pomaderris aspera</i> , <i>Elaeocarpus reticulatus</i> , <i>Hakea eriantha</i> , <i>Acacia dealbata</i> , <i>A. mearnsii</i> , <i>Cassinia</i> sp., <i>Bursaria spinosa</i> , <i>Lomandra longifolia</i> , <i>Lepidosperma</i> sp., <i>Gahnia radula</i> , <i>Olearia lirata</i> , <i>Pteridium esculentum</i> , <i>Goodenia ovata</i> .
C	2	~3 m	50 cm	1	1	<i>E. cypellocarpa</i> , <i>E. globoidea</i> , <i>E. polyanthemos</i> , <i>Pomaderris aspera</i> , <i>H. eriantha</i> , <i>Kimzeia</i> sp., <i>Acacia pycnantha</i> , <i>L. longifolia</i> , <i>Cassinia</i> sp., <i>Gahnia radula</i> , <i>Goodenia ovata</i> .
D	1	0.8 m	30–70 cm	2	2	<i>E. globoidea</i> , <i>E. cypellocarpa</i> , <i>E. consideriana</i> , <i>A. dealbata</i> , <i>Kimzeia</i> sp., <i>Gahnia radula</i> , <i>Stypandra glauca</i> , <i>Cassinia</i> sp., <i>Pteridium esculentum</i> .

Site descriptions

Calling was heard from first, second and fourth order streams (Table 1). All calling sites were within pools in streams with no, or very limited, flow (Fig. 2). The width of streams/pools occupied by calling males ranged from 0.8 m to ~10 m, with pool depth ranging from <5.0 to 70.0 cm (Table 1). The second and fourth order streams where Giant Burrowing Frogs were present have a sandstone base (Fig. 2a).

Calling locations

Giant Burrowing Frogs were observed in their calling position on 14 occasions (Table 2). While calling, all individuals were partially submerged in water (Fig. 1), usually either in shallow water or perched on a prominent rock or log in a deeper pool. One frog was floating while calling. Individuals suspected of being observed more than once occupied different calling locations, sometimes in neighbouring pools.

Influence of weather and stream condition

The role of rainfall in stimulating calling varied. Most detections followed recent rainfall (< 7 days: a product of survey bias), but the two incidental records were obtained 13 and 15 days

Table 2. Calling locations of male Giant Burrowing Frogs.

Description of calling site	No of observations
Edge of stream/pool in small depression or recess and well hidden/sheltered	3
Edge of stream/pool in a relatively exposed location	6
Standing on a prominent rock in stream/pool	3
Standing on a log in log-debris in a stream/pool	1
Floating while calling	1

since rainfall of >5 mm (Table 3). Temperature and humidity were not recorded during these two calling events (in February and August), but at other calling times air temperature ranged from 10.1 to 17.5°C, with 65 to 97% relative humidity. Wind strength was mostly calm during surveys (<10 km/h), but three detections occurred with light breeze (10–20 km/h). Although numerous surveys were undertaken when creeks were flowing moderately, all calling events were at times of no or slow creek flow.



Fig. 2. Calling sites of Giant Burrowing Frogs in the Mount Alfred State Forest. (a) Site B, second order stream; (b) Site D, first order stream.

Calling behaviour

Frequency

Song Meters, programed to operate continuously for three hours after sunset (at three sites), recorded Giant Burrowing Frogs calling on four consecutive nights at one site. These data indicated that calling could be almost continuous, commencing an unknown time prior to sunset and usually averaging 18–19 (range 16–21) calls per minute for virtually the entire recording. This calling rate appeared similar to those of other Giant Burrowing Frogs heard (unless they were disturbed), and is consistent with rates reported by Littlejohn and Martin (1967).

Disturbance

The response of calling individuals to disturbance varied. In some cases observer presence and torchlight caused cessation of calling for several minutes, even at considerable distance (e.g. sometimes >20 m away); calling often recommenced at a slow rate and low volume.

Calling could also be disturbed by vehicles passing nearby. Conversely, some individuals seemed relatively oblivious to disturbance, continuing to call when approached and observed by torchlight.

Calling season and weather influence on detectability

Calling was recorded during five separate months of the year, extending over late Winter, Spring, late Summer and Autumn (Table 3). Rather than reflecting optimal calling periods, these detections were more likely an artefact of local conditions and survey bias, but they do suggest that calling can occur throughout much of the year if conditions are suitable.

Under optimal conditions (no wind) and when frogs were calling from exposed locations, calls could be heard up to 300 m away. However, in less favourable circumstances, calls could be difficult to detect at a distances less than 30 m.

Table 3. The influence of rainfall events on the calling of Giant Burrowing Frogs. A rainfall event is the accumulated rainfall over consecutive rain days (<4 days), calculated from the last rain day. #Song Meters did not detect calling again after this date, *includes an observation of a female close to two calling males on 17/3/12.

Site	Detection date/period	Stream order	No. individuals	Days since last rainfall			Rainfall in previous six months (ml) and percent of long-term average
				>5 mm	>10 mm	>30 mm	
A	13/2/2003	4	2	13	68	75	133 (38%)
B	30/8/2007	2	2	15	15	15	503 (144%)
B	10–11, 16–17/4/2011	2	3	0–1	0–1	18	384 (110%)
C	11/4/2011	2	1	0	0	18	384 (110%)
B	30/9/2011	2	1	0	0	0	279 (80%)
D	11–17/3/2012#	1	3*	1–7	1–7	1–7	609 (174%)

Discussion

Calling and breeding sites of the Giant Burrowing Frog were similar to those described previously; primarily pools with minimal flow from first and second-order streams (Gillespie 1990; Daly 1996; Penman *et al.* 2006c). Of interest was the detection within the fourth order stream; such streams usually have strong flow and therefore do not provide suitable breeding habitat. But this observation was made in an exceptionally dry period when the stream comprised only a series of pools. In contrast, the upper section of a highly ephemeral first order stream also provided suitable conditions. This record followed heavy rainfall (~170 mm), and the calling site was at virtually the highest location that could temporarily hold water (a flooded burrow of a Common Wombat *Vombatus ursinus*; Fig. 2b). Interestingly, the pools dried completely within six weeks, and remained dry for the following 15 months.

These examples demonstrate that Giant Burrowing Frogs are capable of using a diverse range of sites to attempt breeding, but breeding opportunities can be highly variable and often limited temporally, being influenced by factors including rainfall (and other meteorological effects), hydrology, geology and stream order. This is especially applicable to first, third and fourth order streams because they often provide either fast-flowing water or no water. This highlights the importance of streams with sandstone bases that can hold water in pools for long periods, especially some second order streams, allowing increased breeding opportunities and successful tadpole development (e.g.

Daly 1996; Penman *et al.* 2006c). In the Sydney Basin larval life-span extends over 3 to 11 months (Anstis 2013).

Although weather conditions and recent rainfall can stimulate calling (Daly 1996; Penman *et al.* 2006c), the incidental detections of calling 13 and 15 days following rainfall of >5 mm indicate that recent rainfall is not essential for calling to occur. Instead, stream condition/flow appeared critical for stimulating calling. However, despite considerable monitoring during seemingly conducive conditions (appropriate season, limited or no stream flow, temperature >10.0°C, limited wind, recent rainfall >5mm) at sites where Giant Burrowing Frogs had previously recently been active, the detection of calling was rare. Calling activity sometimes differed dramatically between consecutive nights, with frogs calling consistently one night and seemingly being inactive the next, despite apparently similar and suitable climatic conditions. The factors that stimulate calling behaviour and breeding require detailed investigation.

Conservation

Few records of Giant Burrowing Frogs have been obtained in recent decades in Victoria, and the population reported here is currently the only known extant population in the state (Victorian Biodiversity Atlas; Nick Clemann, Graeme Gillespie pers. comm.). Its status has recently been elevated to Critically Endangered in Victoria (DSE 2013). The Mount Alfred State Forest is of particularly high conservation value for the Giant Burrowing Frog, and it is of serious concern, therefore, that potential

threats, notably clear-fell logging and frequent prescribed fires (Penman *et al.* 2005b, 2006a), are regularly undertaken throughout the area. Although the impacts of these practices on the Giant Burrowing frog are poorly understood, implementing additional protective measures throughout the region to conserve important habitat should be a priority. Undertaking studies on the species in the area is a crucial step in helping to inform such future management actions.

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Eighty-nine Years Ago

FROGS IN A FERNERY

Nearly a dozen frogs are at home in my shade-house, and earn their lodging as enemies of slugs and 'slaters', caterpillars, and other pests among the ferns. Several of my pets are Golden Bell-frogs, *Hyla aurea*, one of the handsomest of all known species: others are Common Brown Tree-frogs, *H. ewingii*. The latter are the most confiding; but three of the green and golden frogs, domiciled in the fernery about a year ago, are so tame now that they rarely attempt to jump when touched or taken in the hand. Recent arrivals are wary: The early inhabitants have favourite spots, where they rest during the daytime—their hunting is done after dark. A hanging basket is the 'habitat' of one Brown Tree-frog. It is seen there every day, with green fronds all about it. *H. aurea* is said to include small frogs in its dietary, but, so far, none of the examples in my shade-house has eaten a diminutive neighbour. Tree-frogs especially make interesting pets, and some of the Australian species are dainty and beautiful.

—C. BARRETT

From *The Victorian Naturalist* XLII, p. 234, January 8, 1926

Alcoholic nectar and fermenting yeasts: *Grevillea* 'Robyn Gordon' and *Eucalyptus sideroxylon*

Nicholas Evans¹ and Maria Gibson^{1,2}

¹School of Life and Environmental Sciences, Deakin University,
221 Burwood Highway, Burwood, Victoria 3125

²Contact author

Abstract

The occurrence of ethanol and yeasts capable of fermenting nectar within flowers of *Grevillea* 'Robyn Gordon' and *Eucalyptus sideroxylon* was investigated in an urban situation. Time of day proved crucial for collection of nectar from *E. Sideroxylon*. Ethanol occurred in the nectar of both species but yeasts were cultured only from *G. 'Robyn Gordon'*. *Candida globosa* was identified and is known to ferment all sugars within nectar except xylose. Two species of non-fermenting *Cryptococcus* also were identified including *C. laurentii* which is known to colonise the bronchi of immune suppressed patients. (*The Victorian Naturalist* 132 (5) 2015, 134–138)

Keywords: alcoholic nectar, nectar fermentation, yeasts, *Eucalyptus*, *Candida globosa*

Introduction

Nectar composition varies considerably between species. This may be with respect to sugar composition such as the proportions of xylose, sucrose, fructose and glucose (Ecroyd *et al.* 1995), sugar concentration (Chalcoff *et al.* 2006) and viscosity (Chalcoff *et al.* 2006) as well as other constituents that are present, such as amino acid content. Not all compounds present in nectar are harmless and nectar toxicity is well known. For example, several heavy metals such as copper, zinc and arsenic have been found in nectar and are believed to be transported from roots to flowers by translocation (Ernst and Bast-Cammer 1980). Toxins also can accumulate due to deposition of airborne particles (Ernst and Bast-Cammer 1980). Other chemicals are believed to be synthesised from nectar constituents inside the flower and comprise an array of alcohols, including several benzyl alcohols and ethyl alcohol (ethanol) (Ernst and Bast-Cammer 1980).

Ethanol has been recorded in floral nectar within a variety of studies, e.g. Birtchnell and Gibson (2008); Herzberg (2004); Jackson and Nicolson (2001). It is hypothesised that yeasts colonise the floral cup and ferment the sugars in the nectar to produce the ethanol (Birtchnell and Gibson 2008). Boutroux (1884) produced the first paper on the occurrence of yeasts in nectar and hypothesised that it was not coincidence that they occurred frequently; he con-

cluded that pollinators were potential vectors in the distribution of yeasts in flowers.

Nectar chemistry and plant distribution can influence whether or not yeasts occur in nectar and which species of yeast occur. Several plant species have active resistance to yeasts because of their production of compounds such as benzyl alcohol, cinnamyl alcohol and phenethyl alcohol, known for their anti-fungal properties (Lawton *et al.* 1993). Ascomycetous yeasts frequently occur in flowers with sucrose-dominant nectars but rarely occur in flowers with nectar high in monosaccharides (Mittlebach *et al.* 2015); basidiomycetous yeasts colonise flowers with hexose-dominated nectar (Mittlebach *et al.* 2015). It must be remembered, however, that not all species of yeast found in nectar are capable of fermentation but the number that do occur provide considerable potential for fermentation of nectar of many species (Fleet 2001). For example, *Candida tolerans* occurs abundantly in the nectar of *Hibiscus* spp. and is capable of fermentation (Lachance *et al.* 1999). Melliferous (honey producing) flora can host several different species of yeast, e.g. *Candida* spp., in ethanol laden nectar (Birtchnell and Gibson 2008). Jackson and Nicolson (2001) have shown that xylose, a common nectar sugar, is metabolised to produce ethanol in the Proteaceae.

The observed physiological effects of alcohol on pollinators can be cause for concern. Birtchnell *et al.* (2005) reported hundreds of bees littering the ground after feeding on fermented nectar, the bees showing preference for plants containing the fermented nectar. This also is known for other members of the Hymenoptera, including local species such as native Flower Wasps (Tiphidae) and European Wasps *Vespula germanica* (Hassan 1992; Birtchnell *et al.* 2005). Intoxication of avian pollinators also is widely known.

Ethanol in nectar may have a number of ecological and economic ramifications. Honey production is a multimillion dollar industry for Australia, and potentially could be threatened by this phenomenon through ethanol-induced bee mortality. Fermentation of nectar in the wild appears to be more common in recent years (Birtchnell and Gibson 2008) so it is important to know which plant species produce alcoholic nectar and why. The research presented here is a very small and opportunistic study but contributes to the knowledge gap concerning the occurrence of fermenting yeasts and alcohol content of two common Australian plants. The project aimed to determine:

- whether ethanol occurred in the nectar of the two plants; and
- whether yeasts capable of fermentation occurred in the nectar.

Methods

The study focused on two plant species commonly planted in the Victorian urban environment, thus easily accessible, and well known for their nectar production: *Grevillea* 'Robyn Gordon' and *Eucalyptus sideroxylon*. Both species secrete high volumes of nectar ensuring a plentiful and, therefore, comparatively easy-to-obtain supply.

Nectar was obtained from 30 plants of *G.* 'Robyn Gordon' and 10 plants of *E. sideroxylon*. Selection of sample sites was arbitrary: there was an abundance of the target species in the areas chosen. Ten plants of *G.* 'Robyn Gordon' were sampled at each of:

1. The gardens within Deakin University Burwood;
2. The area defined by the junction of The Boulevard and George Street to Ovens Road and

- Victoria Street in Doncaster, Victoria; and
3. Along the median strip on Dorset Road near the corner of Mt Dandenong Road in Croydon, Victoria.

Five plants of *E. sideroxylon* were sampled at each of:

1. Grounds surrounding the Montmorency Football Club, Montmorency, Victoria; and
2. The Eastern Golf Club, Doncaster, Victoria.

Nectar Collection

Nectar was collected using a modified glass pipette; the tip was heated over a Bunsen burner and pulled to a fine tip. It also was bent to allow easier access to the nectar in the flower. Pipettes were sterilised in sterilisation envelopes using an autoclave. Envelopes were opened immediately prior to collection to minimise the chance of contamination.

Nectar was procured by inserting the sterile pipette into the flower and then sucking the nectar into the pipette using a pipette filler. The nectar was then placed into a sterilised 1.7 mL Eppendorf tube. This was wrapped in Parafilm and placed inside a jar and immediately stored on ice inside a cool box to avoid the potential for fermentation to occur after the sample had been procured. The samples then were refrigerated at 4°C until ethanol and microbial analyses were undertaken, usually the next day.

Grevillea flowers occur in racemes and there are around 40 individual flowers on each raceme. Around 10 of these racemes were sampled on each plant to obtain an adequate amount of nectar from each plant. Racemes were located on separate branches of the plant. Despite sampling 10 racemes, 40 flowers were not necessarily sampled from each raceme; sampling was terminated when an adequate volume of nectar was collected, thus the number of flowers used varied from shrub to shrub.

The *Eucalyptus* nectar was extremely difficult to obtain. Attempts to collect nectar were unsuccessful on several trips, and it was found that the time of day was crucial for success. Subsequently, samples were collected between 0400 and 1200 hours, as this was found to be the time when nectar was most abundant. Between 100 and 200 flowers were sampled per tree to procure sufficient nectar for the analyses.

After collection, the analysis was broken down into two components: microbial analysis and ethanol analysis, which were performed for each nectar sample. This allowed direct comparison of the relationship between presence of yeasts and ethanol.

Microbial Analysis

For each nectar sample (thus each plant), a loop was inoculated with nectar using appropriate sterile technique and streaked onto an agar plate. The medium on the plate was Sabouraud's Dextrose Agar (Oxoid CM41) as this medium is designed for culturing Ascomycota (Sac fungi) and Basidiomycota (Club fungi), thus ideal for culturing yeasts.

Plates were sealed with Parafilm and incubated for 14 days at 18°C inside a culture cabinet. Colonies were then sub-cultured, purified and sent to the Mycology Laboratory, Pathology North, Royal Northshore Hospital (PaLMS), NSW, for identification, where possible (only one per cent of yeast taxa has been identified [Barnett *et al.* 2000]). The remaining nectar was then used for ethanol analysis.

Ethanol Analysis

The presence of ethanol was examined using gas chromatographic mass spectroscopy. Due to the small amount of material available for analysis, injection by auto loader was not feasible, thus samples were manually injected (0.5 μ L) into an Agilent Gas Chromatograph (Agilent Technologies 6890N Network GC system; column; Agilent 19091J-413 HP-5 5% phenyl methyl siloxane capillary, 30 μ m x 320 μ m x 0.25 μ m).

A calibration curve was produced using solutions of ethanol of known concentrations. The chromatograph gives retention times (the time which the vaporised solution takes to move through the column when pushed by energy generated from an internal oven) of volatile substances in the solution. Having the retention times of the known solutions gives a baseline for comparison with unknown solutions of nectar and thus the ethanol concentrations (%v/v) of the nectar samples can be determined.

Results

Microbial Analysis

Yeasts occurred in *G. 'Robyn Gordon'* at all sites examined, although not all shrubs within a site

had yeasts present in the nectar. No yeasts grew in cultures of *E. sideroxylon* nectar. Only three identifiable yeasts were found for *G. 'Robyn Gordon'* (Table 1), two were *Cryptococcus* species and one was a *Candida* (Table 1). Unidentified yeasts were most frequent and generally are referred to as 'environmental' yeasts, probably because most taxonomically known yeasts are linked to human conditions.

Ethanol Analysis

Ethanol was found in the nectar produced by both *G. 'Robyn Gordon'* and *E. sideroxylon* but the number of trees for which this occurred varied from site to site (Figs. 1 and 2). High variability of ethanol concentration occurred between sites (Figs. 3 and 4) although this was not statistically significant (*G. 'Robyn Gordon'*: $F=1.03$; $df=9$; $p=0.44$. *E. sideroxylon*: $t=-1.4$; $p=0.09$).

Discussion

Only one of the three yeasts identified in this study, *Candida globosa*, is known to ferment sugars found in nectar. Indeed, it can ferment all nectar sugars with the exception of xylose (Barnett *et al.* 2000). This yeast occurred in a nectar sample containing alcohol, thus could have been responsible for fermentation of the nectar. *Candida globosa* also has been found in fermenting sugar cane in Spain and in fermenting fruiting bodies in the UK. This study has presented the first record of its presence in nectar of Australian flora.

Cryptococcus laurentii is a non-fermenting yeast and has been found on species of the Proteaceae and Myrtaceae in Australia, suggesting a common affinity with taxa of the same lineage (Barnett *et al.* 2000). It also is known to have a pathogenic relationship with humans, colonising the bronchi of immune suppressed patients (Barnett *et al.* 2000). *Cryptococcus albidus* was another non-fermenting yeast. Most yeasts found in this study were not able to be identified and were broadly categorised as environmental yeasts. This came as no surprise as less than 1% of the yeast taxa have been classified (Mrak and Praff 1948; Barnett *et al.* 2000). Therefore, it is hard to ascertain the role of environmental yeasts in nectar fermentation, although several of these were found in the presence of alcoholic

Table 1. Yeasts of *Grevillea* 'Robyn Gordon'.

Yeast species	Number of plants in which yeasts occurred (n=30)
Unidentified (environmental) yeasts	17
<i>Cryptococcus laurentii</i>	4
<i>Cryptococcus albidus</i>	3
<i>Candida globosa</i>	1

nectar. Further microbial investigation would have allowed more conclusive results but this study was of an opportunistic nature and funding was very limited.

Flowers of everal trees contained ethanol but no yeasts were isolated. Yeasts are only one of various types of micororganisms that could be responsible for fermentation of nectar. Several anaerobic bacteria and lignocellulosic fungi also have the potential to ferment sugars occur-

ring in nectar. Certain *Eschericha* and *Salmonella* species can ferment sugars and are widespread in the environment. They also have been found to occur in nectar and honey (Snowdon and Cliver 1995). A number of trees examined contained bacterial and fungal growths in the presence of alcoholic nectar but, as this study examined only yeasts, these growths were not identified.

Nectar composition differs from species to species and even within a species. This can be due to a number of factors including temperature, nutrient availability, water availability and target pollinator species (Perret *et al.* 2000; Baker and Baker 1983). It is hypothesised that the sugar composition of nectar may influence the yeast or other microorganism distribution, thus affecting fermentation potential (Nicolson and Van Wyk 1998; Freeman and Wilkin 1987). Future studies could examine sugar composi-

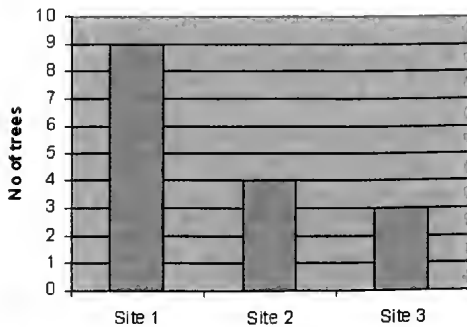


Fig. 1. Number of *Grevillea* 'Robyn Gordon' shrubs with ethanol present in the nectar. Site 1: Deakin University (n=10); Site 2: Doncaster (n=10); Site 3: Croydon (n=10).

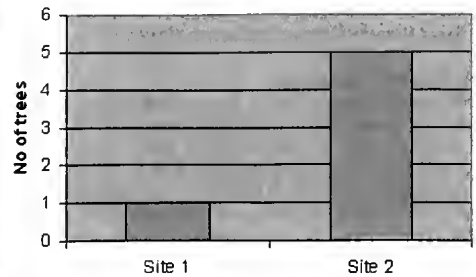


Fig. 2. Number of *Eucalyptus sideroxylon* trees with ethanol present in the nectar. Site 1: Montmorency (n=5); Site 2: Doncaster (n=5).

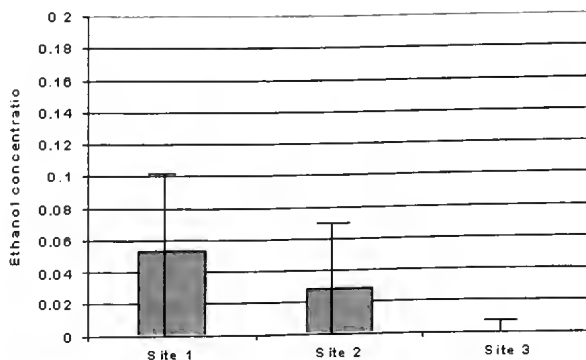


Fig. 3. Mean and Standard Deviation of ethanol concentrations in *Grevillea* 'Robyn Gordon' nectar. Site 1: Burwood, Site 2: Doncaster, Site 3: Croydon.

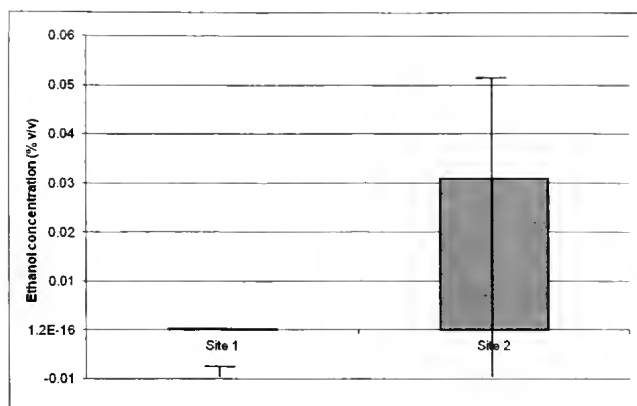


Fig. 4. Mean and Standard Deviation of ethanol concentrations in *Eucalyptus sideroxylon* nectar. Site 1: Montmorency, Site 2: Doncaster.

tion, identify other microorganisms present in nectar and, possibly, fermentation experiments under laboratory conditions to determine whether specific microorganisms could ferment sugar under different humidities and temperatures—the two environmental variables considered to trigger fermentation in floral cups (Birchinnell and Gibson 2008).

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Premature opening and dimorphism in *Hakea decurrens* (Proteaceae) follicles: a bet-hedging regeneration strategy?

Marilou Meehan^{1,2}, Alexander Keirsten-Wakefield¹, Madeleine Cowan¹
and Juli Atkinson¹

¹Department of Ecology, Environment and Evolution, La Trobe University, Bundoora, Victoria 3086

²Corresponding author

Abstract

Hakea decurrens (Proteaceae) is generally regarded as a strongly serotinous species, with woody follicles opening only after fire; however, field observations indicate that follicles do open in the absence of fire. This study examined whether premature follicle opening served as a bet-hedging regeneration strategy, by which plants could recruit during protracted inter-fire periods. Two follicle morphs were identified: a thinner 'slug', and a thicker, more robust 'snail'. 'Slug' morphs were always observed open, while 'snail' morphs varied in the proportion of open follicles across several study areas with differing time-since-fire, highlighting a moderate correlation between the number of open 'snail'-shaped follicles and older trees. The proportion of open follicles was compared with that of *Hakea eriantha*, a species which occurs in very similar vegetation types to *H. decurrens*. Fewer open follicles were observed in this species. There was no sign of successful *H. decurrens* recruitment during inter-fire periods, indicating that premature follicle opening is not reproductive bet-hedging. Lack of inter-fire recruitment, as well as observations of webbing and insect larvae within a majority of 'slug' follicles, led to the formation of a new hypothesis which highlights seed abortion and granivory as potential key drivers behind follicle dimorphism and premature follicle opening in *H. decurrens*. (*The Victorian Naturalist* 132 (5) 2015, 139–146)

Keywords: fire, serotiny, granivory, fruit, herbivory, seed, senescence

Introduction

Serotiny has evolved independently and repeatedly in a number of widely separated plant taxa (Simon *et al.* 2009). In serotinous species, plants retain their seeds within protective woody fruits or cones in the canopy, delaying seed release until triggered by an environmental stimulus (LeMaitre 1985; Midgley and Enright 1999). Serotiny is a convergent plant trait thought to be influenced by selective forces such as fire, soil nutrient availability, and seed predation (Clarke *et al.* 2012). Within a single plant community, the degree of serotiny can differ between species, and sometimes even within species, typically dictated by the environmental gradients along which these individuals might occur (Enright and Goldblum 1998). Weakly serotinous species tend to retain seeds for shorter periods and release seed spontaneously in comparison to their strongly serotinous counterparts. Some strongly serotinous species can open follicles upon reaching reproductive maturity after five years (Groom and Lamont 1997), although most tend to retain their seed

for periods upwards of ten years—sometimes indefinitely if external triggers are absent or fail to cue seed release. Strong serotiny is a trait synonymous with obligate pyriscence (Lamont *et al.* 1991), and tends to lead to trade-offs in seed production, resulting in fewer seeds per follicle (Cramer and Midgley 2009).

Varying degrees of serotiny are expressed in the genus *Hakea* (Groom and Lamont 1997), with some species believed to recruit only after fire as obligate reseeders. *Hakea decurrens* and *Hakea eriantha* are two such reseeding species that rely entirely on seeds stored within their canopy seed banks for post-fire recruitment (Enright and Goldblum 1998). As both species are typically thought to be strongly serotinous, these species are vulnerable to local extinctions if fire intervals are shorter than the time taken for them to reach reproductive maturity (Bradstock *et al.* 1997; Ooi *et al.* 2006). Interestingly, follicles of *H. decurrens* appear dimorphic, present as either 'slug' or 'snail' morphs (Fig. 1), and open follicles have often been observed in

the absence of fire. It is unclear why two follicle morphs exist; this may be associated with an alternative recruitment strategy, where some seeds are released during protracted inter-fire periods. This would, in turn, indicate a lesser degree of serotiny in *H. decurrens*. However, it is also possible that seeds are merely being aborted early.

Enright and Goldblum (1998) recorded instances of inter-fire recruitment within *H. decurrens* stands where time-since-fire was between 24 and 28 years. A correlation was found between older stand ages, greater instances of adult mortality, increased seed release and the incidences of severe drought (Enright and Goldblum 1998). This leads to the possibility that environmental stresses other than fire (such as drought, disease and senescence) may play a role in inter-fire recruitment, which is a potential indication that *H. decurrens* may employ a 'bet-hedging strategy', commonly witnessed in 'weakly serotinous' species (Lamont *et al.* 1991), in order to maintain population numbers during protracted inter-fire periods (Clarke *et al.* 2012; Whelan *et al.* 1998). However, successful seedling establishment may not always occur during cases of extreme environmental circumstance or stress (Groom and Lamont 1998).

In order to determine whether a bet-hedging strategy is indeed at play, this study aimed to quantify the degree of inter-fire recruitment of both *H. decurrens* and *H. eriantha*—two obligate reseeders that occur in the Cape Conran hinterlands of East Gippsland, Victoria. It was predicted that bet-hedging would increase as time-since-fire increased, and consequently more follicles would be open on older plants. This study also aimed to discover if follicle dimorphism and premature follicle open-

ing in *H. decurrens* was a component of this bet-hedging strategy. The expectation was to observe a difference in the proportion of open and closed follicles between 'slug' and 'snail' morphologies.

Methods

Study Species

This study focused on two native, strongly serotinous and obligate reseeders of the Proteaceae family: *Hakea decurrens* subsp. *platytaenia* and *Hakea eriantha*.

There are currently three subspecies recognised under *Hakea decurrens*. These are *H. decurrens* subsp. *decurrens*, *H. decurrens* subsp. *physocarpa*, and *H. decurrens* subsp. *platytaenia*—spanning the dry sclerophyll forests, woodlands and heaths of New South Wales, Victoria and Tasmania on sandy and rocky soil (Barker *et al.* 2000). The subspecies of focus within this study, *H. decurrens* subsp. *platytaenia*, is present along the coastal heathlands of eastern Victoria, south-east NSW and the Bass Strait Islands (Barker *et al.* 1995), and appears as a spreading shrub or small tree up to 5 m in height. This species produces ovoid horned and beaked follicles which appear dimorphic, with individual follicles found to vary between 18 to 35 mm in length and 14 to 36 mm in width on a single plant (Barker *et al.* 2000). Plants become reproductively mature at three years and senescence begins at approximately 24 years (Enright and Goldblum 1998).

H. eriantha is a less widespread species, restricted largely to wet sclerophyll forests and woodlands of coastal New South Wales and Victoria. Plants range from shrubs to trees of 10 m high and produce smooth beaked oblong-ovoid follicles 20 to 30 mm long and 15 mm wide (Barker *et al.* 2000), which closely resemble *H. decurrens* 'slug' follicles. Outside Victoria, *H. eriantha* also has been recorded as lignotuberous (Baker *et al.* 1996).

Study Sites

In order to compare the degree of inter-fire recruitment occurring in different-aged stands of *H. decurrens*, four areas with differing time-since-fire (1981, 2001, 2005 and 2010—known year of last burn) were identified in the Cape Conran hinterlands of East Gippsland, Victoria



Fig. 1. Sketch of *Hakea decurrens* follicles, showing a 'slug' (left) and 'snail' (right). The follicles of *Hakea eriantha* resemble the 'slug' morphology of *Hakea decurrens*. (Scale: x1.1 magnification).

(Fig. 2). The *H. decurrens* subsp. *platytaenia* sites were located along Marlo–Cabbage Tree and Cabbage Tree–Conran Roads, all roughly within 2 kilometres of one another, while the *H. eriantha* site was located along Palm Track, approximately seven kilometres north-west of the other sites.

The Cape Conran area has a mean minimum annual temperature of 12°C and a mean maximum of 19°C, and receives an average of 964 mm of rainfall per year (Bureau of Meteorology 2014). Soil types found within the coastal park are generally sandy in areas that support forest vegetation (Cape Conran Coastal Park Management Plan 2005).

Sampling Method

Within each of the four fire histories, 10 *H. decurrens* plants were selected randomly by blindfolding and disorientating a researcher, and then having them select a random direction in which to locate the nearest plant. Of the 10 plants selected within each fire zone, the first five were used to mark the centre of a circular quadrat with a radius of 1.5 m. The basal trunk diameters of all conspecific saplings and trees within this area were measured to evaluate the extent of recruitment in the absence of fire.

Basal diameter has been considered a reliable proxy for assigning plant age classes (Perryman and Olson 2000).

Each of the ten randomly selected focal plants per fire zone was used to assess the extent of follicle opening in the inter-fire period. All follicles (or up to 100 on older plants) were counted to determine the proportion of open versus closed follicles per tree. Due to the appearance of follicle dimorphism in *H. decurrens*, each follicle counted as either open or closed was also scored as either a 'slug' (a thin and small follicle) or a 'snail' (robust and comparatively large) (Fig. 1) in order to assess whether one morph was more likely to open than the other in the absence of fire.

In order to explore whether the degree of inter-fire recruitment is variable within the genus, a stand of *H. eriantha*, unburnt since 1981, was visited to compare against the *H. decurrens* stand of the same age. *H. eriantha* was chosen as a suitable comparative species in this study because it is also an obligate reseed and occurs in the same coastal heath and woodland environment as *H. decurrens* (National Herbarium of NSW 2014); however, it does not exhibit the same 'slug'/'snail' follicle dimorphism. The

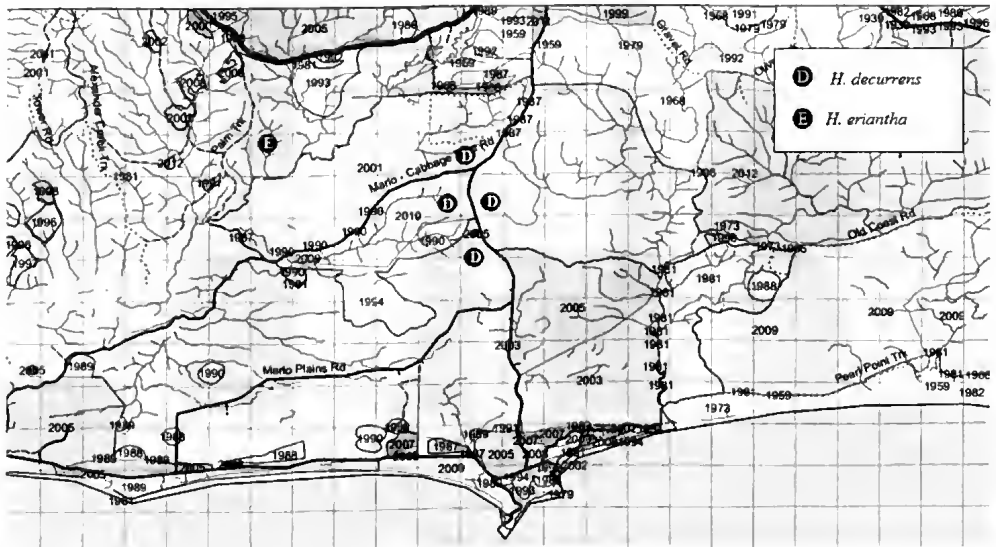


Fig. 2. Map of Cape Conran fire history. Dots represent study sites of four different fire zones (1981, 2001, 2005 and 2010) for *Hakea decurrens* and one *Hakea eriantha* (1981). (Map source: DEPI).

proportion of open and closed follicles of the 10 *H. eriantha* plants was scored in the same manner as *H. decurrens*.

Statistical Analysis

T-distribution confidence intervals were calculated for the proportion of open 'slugs' and 'snails' at each fire site, and in *H. decurrens* and *H. eriantha* in total. The correlation between basal diameter and number of open follicles of *H. decurrens* was examined using linear least squares to produce a line of best fit.

Results

'Slugs' were always open at all sites (Fig. 3). The 1981 fire cohort had a statistically significantly higher proportion of open 'snails' than the 2001 and 2005 cohorts; all other differences were not significant. A trend of increasing proportion of follicles open with increasing time-since-fire was observed, except in the 2010 site. This is likely due to the smaller follicle sample size, and correspondingly higher variance, in the 2010

fire cohort, in which individual plants carried few follicles.

Size class distributions of *H. decurrens* were narrow. Notably, the 1981 site contained many larger individuals, but no plants with a base diameter less than 10 cm. This indicates pulse recruitment, and thus no inter-fire recruitment in the near vicinity of reproductive plants, despite the opening of follicles (Fig. 4).

There was a trend of increasing proportion of open 'snails' with increasing basal diameter ($R^2 = 0.40$, Fig. 5). This shows a moderate positive correlation, indicating that open 'snails' accumulate over time. A large contribution to the variance came from the 2010 fire site; exclusion of this data would increase the strength of the relationship.

H. eriantha had significantly fewer open follicles than *H. decurrens* (Fig. 6). *H. eriantha* also lacked the differing follicle morphologies found in *H. decurrens*.

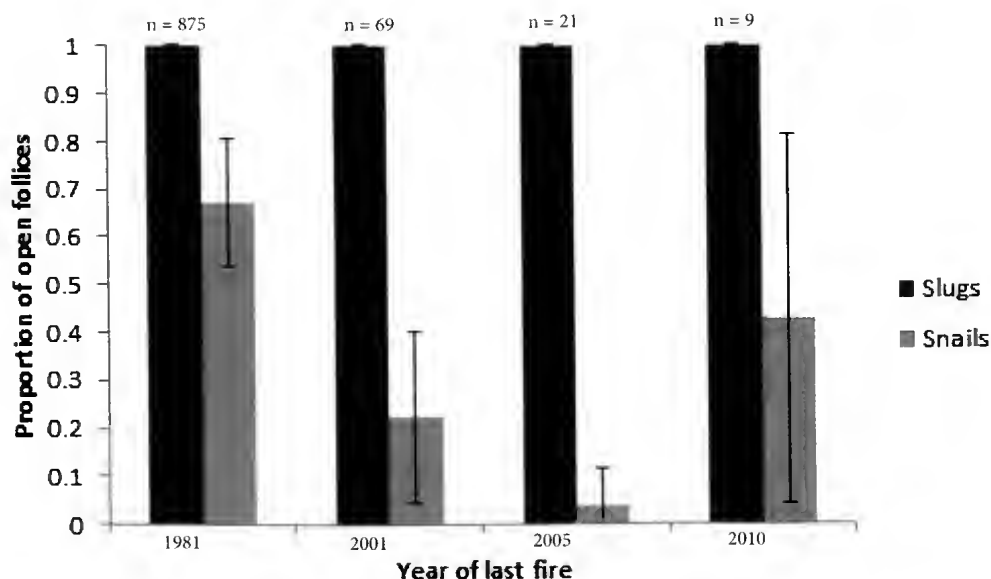


Fig. 3. Proportion of open 'slug' and 'snail' follicles on *Hakca decurrens* in different fire sites. Error bars show the 95% confidence interval. Sample sizes for 'slugs' are 75, 58, 7 and 8 for 1981, 2001, 2005 and 2010, respectively. Sample sizes for 'snails' are 875, 69, 21 and 9 for 1981, 2001, 2005 and 2010, respectively.

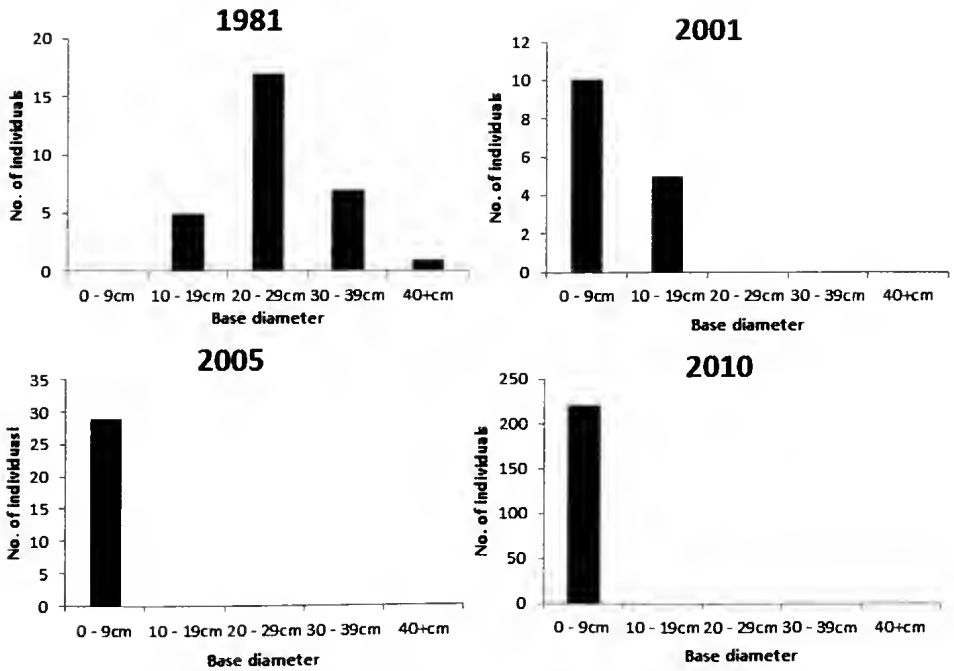


Fig. 4. Size class distributions for *Hakea decurrens* fire sites, showing narrow distributions of age classes.

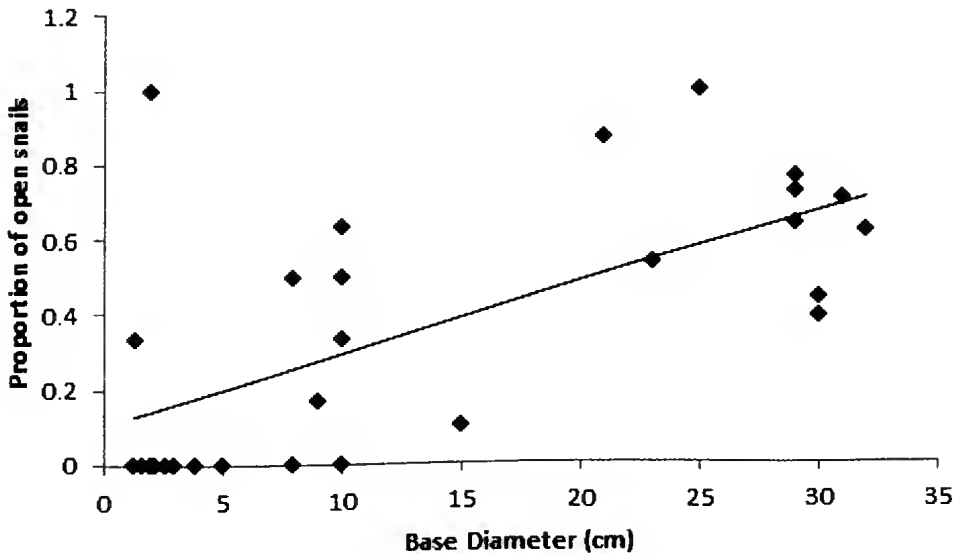


Fig. 5. Proportion of open 'snails' and *Hakea decurrens* basal diameter with line of best fit (least squares). $R^2 = 0.40$.

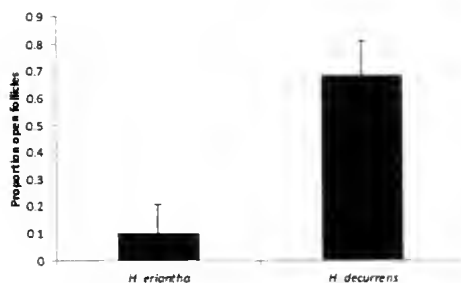


Fig. 6. Proportion of open follicles in *Hakea eriantha* versus *Hakea decurrens*, of the 1981 cohort. Error bars are 95% confidence interval. A total of 383 follicles were examined in *H. eriantha*, versus 1122 follicles in *H. decurrens*.

Discussion

The original premise for this study was to investigate whether bet-hedging was an underlying recruitment strategy driving premature follicle opening in *H. decurrens*. As expected, the proportion of open follicles was found to increase as time-since-fire increased, and there was a significant difference between the proportion of open 'slug' and 'snail' morphs, with 'slugs' open 100% of the time. However, there were no signs of successful recent recruitment in older stands, from which it is inferred that inter-fire recruitment had not occurred in any of the four fire zones. Premature follicle opening thus appears not to be a bet-hedging strategy.

There is an indication that open 'snails' accumulate over time, as the data points to a moderate correlation between the number of open 'snail'-shaped follicles and older plants. In older stands, where these observations were recorded, there seemed to be greater instances of adult mortality and follicles could be expected to open as plants senesce. Fewer open follicles were observed on *H. eriantha* suggesting that, whilst both species are serotinous, the degree of serotiny varies substantially between the two species occurring in very similar vegetation types.

As 'slugs' were always found to be open, it is possible that they represent follicles that were aborted before becoming 'snails'. Plants in the 2010 fire site possessed open follicles of both morphologies, despite being less than four years old. These seeds were unlikely to have lost viability due to age, implying that there must be some other cause of seed leakage.

Groom and Lamont (1997) proposed that strong serotiny in *Hakea* species was linked with significantly thicker and denser follicles than fruits of weakly serotinous species. Whilst this trait was evidently adapted for the protection of seed during fire, it may also confer the additional fitness benefit of reduced seed predation (granivory). Given this hypothesis, it seems reasonable to infer that the more robust 'snail' morphs are the desired adaptive trait in *H. decurrens*, providing protection for seeds within thicker, denser follicles. Midgley (1991), however, argues that follicle size variation is more a product of seed mass within the follicle, which determines the follicle size, and thus the degree of serotiny.

Whilst contentious, there is a convincing body of evidence that indicates granivory is a major driver of serotiny evolution. Bradshaw *et al.* (2011) described the fossil remains of a *Banksia* species from the Oligocene with evidence to suggest that predation by birds, insects and mammals may have been favouring the evolution of woody fruits for millions of years. Observations by Naser (1968) found that moth larvae are more likely to die from predation, starvation and dehydration whilst attacking a strongly serotinous *Hakea* species from the outside. Earlier experimental evidence, collected over three consecutive years by Gordon (1992), described a similar interaction between seed moths and a *Hakea* species. His data indicated that whilst seed destruction may not always be severe, targeted and substantial predation can lead to a reduction in the accumulated canopy seed by up to 64%. As such, Zammit and Westoby (1988) also considered that the increased metabolic cost of producing thick and woody follicles for the protection of nutritious and limited seed in many Proteaceous taxa may account for such a selective pressure, particularly since they believe that the alternative strategy may very likely have resulted in an increased degree of predation within the seed bank.

Consequently, the correlation between thicker, denser follicles and strong serotiny may be explained by granivory pressure. In the case of *H. decurrens*, it seems external seed predation is not the only type of predation occurring. In the field, observations of webbing and the presence of insect larvae within a

large majority of the open 'slug'-shaped follicles led to a new hypothesis: 'slug' morphs are a response to granivory and the result of early seed abortion, whereby the plant cuts its losses after detection of unviable seed, produced when floral infestation by a parasitic insect occurs.

This hypothesis may explain supposed follicle dimorphism and premature follicle opening in *H. decurrens*. True follicle dimorphism in the *Hakea* genus has been described formally only in the locally-occurring *Hakea nodosa*, which produces two distinct follicle morphs, one which remains closed while attached to the bush and the other which opens while still attached (Barker *et al.* 1996). However, signs of invertebrate interference and seed abortion in the case of *H. decurrens* suggest that the so-called dimorphism observed during this study may actually be a symptom of granivory or abortion of seeds. It is still unclear whether the presence of insect larvae within open 'slug' follicles is direct evidence that floral infestation has, in fact, occurred. It is quite possible that 'slug' morphs are merely aborted seeds and as such, follicles open once nutrients from the plant are no longer received, creating suitable habitats for insect larvae after the fact. Nonetheless, the presence of webbing or insect larvae within 'slug' morphs collected or observed in the field remained consistent across all fire zones, leading to the conclusion that granivory is likely occurring, albeit at a much earlier stage of follicle development. The fact that follicle dimorphism does not occur in *H. eriantha* may also indicate that some type of symbiotic relationship may have evolved between *H. decurrens* and the insect species which inhabit 'slug' follicles. However, further studies are required to ascertain whether this is plausible.

Conclusion

As this study was designed to determine whether bet-hedging was a recruitment strategy adopted to maintain population numbers during protracted inter-fire periods, many of the interesting observations from this study still remain untested. Exploring the possibility of early seed abortion or determining whether granivory is the major driver of follicle dimorphism and inter-fire follicle opening is a compelling hypothesis that deserves further consideration in

future studies. A more in-depth study to closely observe 'slug' follicles for the presence of larvae or webbing, as well as genetic analysis of the larvae occupying open follicles, should also be conducted to determine whether the insects targeting *H. decurrens* are a single species or not. A single species or closely related group of insects could expose interesting evolutionary relationships at play. Along with this, a comparative analysis between *H. decurrens* and *H. eriantha* should include the examination of other environmental stresses, such as drought and disease, to determine why such a high proportion of *H. decurrens* follicles are aborted early.

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One Hundred and One Years Ago

Exhibition of wild-flowers

As usual, the October meeting of the Club was devoted principally to the annual exhibition of wild-flowers; but seldom in the long series of displays made by members of the Club have the flowers been representative of such a small portion of Victoria as on the present occasion. Owing to the exceedingly dry winter and spring in the central and northern portions of the State, most of the flowers were obtained from the south and east of the metropolis. A noticeable effect of the dry weather was the poor display of *Tetratheca ciliata*, Pink Eyes, bunches of which usually brighten the exhibitions. Fortunately, fine displays from cultivated plants were made by Mr. J. Cronin, Director of the Melbourne Botanic Gardens; Mr. E. E. Pescott, Principal of the Horticultural Gardens, Burnley; and a smaller number by Mr. Hugh Anderson, of Tooronga House, Hawthorn, which, besides providing exhibits to look at, demonstrated the fact that a large number of our native plants can be successfully cultivated in our gardens if given the necessary attention.

The collection from the Botanic Gardens comprised blooms of fifty species of Victorian plants, among which may be mentioned : — *Leptospermum myrsinoides*, Pink Tea-tree; *Livistona australis*, Australian Cabbage-Palm; *Kunzea cordifolia*, White Kunzea; *Cassia australis*, Southern Cassia; *Callistemon salignus*, Willow Bottle-brush; *Stypandra glauca*, Blue Spray; *Calythrix Sullivani*, Grampian Fringe-Myrtle; *Bauera rubioides*, Wiry Bauera; *Prostanthera melissifolia*, Balm Mint-bush; *P. nivea*, Snowy Mint-bush; *Phebalium Billardieri*, Satin-wood Phebalium; *Swainsona Greyana*, Pink Swainsona; *Clematis aristata*, var. *Dennises*, Pink-flowered Greater Clematis; and *Boronia pinnata*, Feathery Boronia.

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Establishing indigenous vegetation in degraded natural or constructed wetlands*

Damien Cook

Rakali Ecological Consulting, PO Box 188, Chewton, Victoria 3451. Email: damien@rakali.com.au

Abstract

Increasing the cover and diversity of native wetland plants in constructed or degraded natural wetlands can improve their habitat value, water treatment capability and aesthetic appeal. This article describes a method for successfully establishing indigenous aquatic vegetation within wetlands. (*The Victorian Naturalist* 132 (5) 2015, 147-153)

Keywords: Wetlands, restoration, revegetation, aquatic plants

Introduction

In Victoria, 50% of the area of natural wetlands has been destroyed since European occupation (DCE 1992). Restoring degraded natural wetlands, or establishing indigenous vegetation in constructed wetlands, provides an opportunity to increase the habitat available for a wide variety of plants and animals that have become rare because of habitat loss. These wetlands also provide places where people can learn about and enjoy natural ecosystems. For example, the 48 hectares of revegetated wetlands and terrestrial areas at the Waterways (Fig. 1), near Edithvale in Melbourne's south-eastern suburbs, have attracted over 100 species of birds, including the rare Magpie Goose, and seven species of frogs. They also support over 220 species of indigenous plants, including 14 rare and threatened species such as the nationally vulnerable Swamp Everlasting *Xerochrysum palustre*. This area is regularly visited by nature lovers and other members of the public.

To provide water quality improvement function in constructed wetlands or restore the biodiversity of degraded natural wetlands, it is necessary to deliberately establish or increase the abundance and diversity of indigenous plant species. The establishment of vegetation within wetlands can present a number of unique challenges. The information and techniques that can be used to overcome these challenges presented here have been developed through 15 years of practical experience in growing and successfully establishing wetland vegetation. Clewell *et*

al. (2005) provide more detailed guidelines for developing and managing ecological restoration projects.

Methods

The methodology for establishing wetland vegetation involves the following steps:

1. Determine the physical, chemical and biological characteristics of the wetland environment to be revegetated
2. Determine the function (habitat, water treatment etc.) and characteristics (height, density, appearance etc.) of the vegetation to be established
3. Set goals for plant establishment
4. Select appropriate species
5. Source plant material
6. Plant and establish desired species
7. Maintenance
8. Monitoring

Critical aspects of each of these points will be discussed in detail for both natural degraded and constructed wetlands. It should be noted that to improve the health and diversity of vegetation in natural degraded wetlands it is first necessary to address or manage degrading processes such as grazing, cropping, altered hydrological regimes or artificially high nutrient inputs. Indeed, this may lead to plant re-establishment without the need for further intervention.

1. Determine the physical, chemical and biological characteristics of the wetland environment to be revegetated

The composition, structure and distribution of vegetation that will grow in a wetland are de-

* This paper was presented at the Biodiversity Symposium of the FNCV 'Water and Biodiversity' in 2013.



Fig. 1. Revegetated habitat wetland at the Waterways. Dominant species include Running Marsh-flower *Vilarsia reniformis* and Fine Twig-sedge *Baumea arthropphylla*.

terminated by a combination of physical, chemical and biological factors. An understanding of these factors and how they influence vegetation is essential when determining what plants to establish and where to put them.

Inundation depth, duration, frequency and seasonality have a major influence on what species grow where within wetlands. When planning wetland revegetation, it is helpful to map out areas in terms of their hydrological habitat, which requires topographic information combined with data on known or modelled wetting and drying cycles. A schematic cross-section of these zones and the plants which grow in them is a useful tool. A combination of the following generalised habitats may be found around most wetlands and usually support distinct associations of plant species.

i) The interface zone between the wetland and surrounding terrestrial vegetation: is above the normal high water level but soil moisture is influenced by lateral seepage and may be saturated during winter and inundated for short periods after rain events. The plant species occurring in this ecotonal environment vary from sedges, rushes, grasses

and other herbaceous plants to shrubby thickets of Tea-tree (*Melaleuca* and *Leptospermum* species) in higher rainfall areas or *Lignum Duma florulenta* in low rainfall areas.

- ii) Meadow zone: often has a very shallow gradient and is inundated by up to 100 mm at normal high water level and dries out seasonally. Drying out assists in the nutrient cycling process. Common life forms of this habitat include erect emergent sedges (*Eleocharis*, *Carex*, *Cyperus* and *Baumea* species), rushes (*Juncus* species), grasses (*Poa* and *Amphibromus* species) and tufted or spreading herbs (*Lobelia*, *Goodenia* and *Eryngium* species).
- iii) Shallow Marsh zone: is inundated from 100 mm up to 250 mm at normal high water level and usually drying out completely during summer and autumn or drought. Common lifeforms include erect emergent sedges (*Eleocharis*, *Carex*, *Cyperus*, *Bolboschoenus* and *Baumea* species), rushes (*Juncus* species), grasses (*Glyceria* and *Amphibromus* species) and amphibious herbs with floating or emergent leaves (*Myriophyllum*, *Triglochin*, *Orn-*

duffia, *Marsilea*, *Ranunculus* and floating-leaved *Potamogeton* species).

- iv) Deep Marsh zone: is inundated from 250 mm up to 1000 mm at normal high water level, the Deep Marsh zone is generally permanently inundated but may dry out completely during drought. Common lifeforms include tall erect emergent sedges (*Eleocharis sphacelata*, *Baumea articulata* and *Schoenoplectus tubernaemontani*) and aquatic herbs with floating, emergent or submerged leaves (*Myriophyllum*, *Triglochin*, *Ornduffia*, *Nymphoides* and *Potamogeton* species) and free floating species such as *Azolla* or duckweeds.
- v) Open Water zone: is greater than 1000 mm deep and generally supports no emergent vegetation. This zone may support submerged aquatic plants such as Eel Grass *Vallisneria spiralis* and Pondweeds *Potamogeton* species.

Wetting and drying cycles are critical for maintaining the health of wetland vegetation (Butcher 2008). Reduction in water level or complete drying out due to evaporative loss over dry seasons, termed draw down, is important in the cycle of nutrient breakdown and release. It maintains macrophyte species composition and structure and promotes the flowering, seed production and recruitment of many species of plants. It also provides the mud flat habitat preferred by many species of wetland birds such as Dotterels, Plovers and Sandpipers.

Wetlands that maintain high water levels year round due to artificial inputs of stormwater or irrigation run-off often become dominated by vigorous, warm-season growing plants such as *Typha*, *Phragmites* and *Schoenoplectus* species, which can outcompete many other species and reduce biodiversity (Roberts and Marston 2011). In natural degraded wetlands the act of restoring natural wetting and drying cycles may be enough to stimulate the re-establishment of many plant species.

Constructed wetlands should be designed so that they provide meadow, shallow marsh and deep marsh habitats with appropriate wetting and drying cycles. There are many examples of constructed wetlands where plant establishment has failed or been poor because imposed hydrological regimes are just too different from natural cycles. This problem can be overcome

by innovative design informed by an understanding of wetland ecology.

For example, constructed wetlands can be designed to have distinct ponds or cells separated by overflow weirs. One pond is the primary treatment cell through which all incoming water passes. During times of normal low flow, this is the only cell through which water moves. Water flows into other cells only on occasions after rainfall events in which water levels in the primary cell rise high enough to overtop their overflow weirs. In this way, close to natural wetting and drying cycles can be achieved in these normally still water ponds, and diverse vegetation can be established within them. The primary treatment pond may experience a fairly artificial water regime, with near constant flows, and therefore supports only fairly simple riparian vegetation.

Constructed wetland inlets and outlets should be designed so that floodwaters entering wetlands are retained only for a short time in order to prevent plants from drowning or becoming covered in algae. In areas with large catchments it may be useful to have a bypass channel which allows high flows around the wetland to prevent regular pulsing of water levels during the plant establishment phase. An ability to raise and lower levels by the use of weirs and sealable outlet pipes can be useful for plant establishment and management purposes such as weed control or facilitating summer draw down.

Salinity, pH, turbidity and substrate characteristics

The physical and chemical characteristics of water and soils within wetlands are also important in determining what will grow in a wetland (Roberts and Marston 2011). While some plant species seem to thrive under a wide range of environmental conditions, many others do well only within a particular range of salinity, pH and turbidity. It is important to note that some of these characteristics may vary widely within a wetland depending on seasonal changes; for example, a wetland may be quite fresh during wet periods but become quite saline in dry times.

Soil nutrient levels and water holding capacities also play a major role in determining plant species composition within wetlands. Wetlands occurring on heavy clay soils will support flora very distinct from those occurring in sandy

areas, even if their hydrological regimes are similar. Plant establishment within constructed wetlands is greatly enhanced if at least 150 mm of local topsoil is spread in areas in which aquatic plants are desired.

Wave action

Wide, open bodies of water with a large surface area can be subject to intense wave action caused by strong winds. This wave action can cause bank erosion and prevent the establishment of many plant species. Such water bodies, unless they have very gentle bank gradients (i.e. of a higher ratio than 1:30), will usually support only simple vegetation communities. Wave action can be reduced in constructed wetlands by decreasing the length (fetch) of a water body in the direction of prevailing winds, using islands or shallowly submerged bunds.

2. Determining the function and characteristics of the vegetation to be established

The primary functions of a constructed wetland will determine what kinds of plant species and associations will be desirable to establish within it. Wetlands with the primary function of water treatment will need large areas of dense emergent macrophytes that will intercept water flow, facilitate deposition of suspended sediments, and support a large surface area of biofilm (consisting of fungi, algae and bacteria). The emergent macrophytes which are best suited to this role are those that grow rapidly, take up substantial quantities of nitrogen and phosphorus, and do not die off in cool seasons, for example *Baumea articulata* and *Eleocharis sphacelata*. They will also require deep areas with no emergent vegetation to allow maximum sunlight penetration so that ultraviolet radiation can kill undesirable bacteria.

Constructed wetlands with the primary function of wildlife habitat should be planted with the plant species whose characteristics provide for the habitat requirements of the wildlife species for which they are being designed. For example, dense stands of Water Ribbons *Triglochin procerum* and submerged beds of Eel Grass and Pondweeds are the favoured habitats of the Growling Grass Frog *Litoria raniformis* and Green and Gold Grass Frog *Litoria aurea*, as they provide ideal surfaces on which to bask and dense cover in which to hide from predators.

Wetlands with the primary role of increasing aesthetic values should be planted with attractive species that produce massed displays of flowers, such as *Ornduffia* or *Nymphoides* species, or have interesting foliage colour and texture, such as *Myriophyllum* and *Triglochin* species. The use of tall, vigorous emergent sedges in such areas is to be avoided as they will ultimately obscure views into the wetland. Well-designed or managed wetlands can perform all of the above functions as long as areas with conflicting requirements are kept separate. Visiting intact remnant wetlands is the best way to see how this can be achieved.

Retain, enhance and interpret existing ecological, landscape and cultural features, such as large old trees, remnants of native vegetation and sites of archaeological or historic significance. These are valuable assets that will be of interest to the local community and help to create a unique sense of place.

3. Setting goals for plant establishment

How densely a wetland is planted will determine how rapidly aquatic vegetation is established, and the cost of planting and weed maintenance. The Interface, Meadow and Shallow Marsh zones are those most susceptible to weed invasion in the early phase of wetland vegetation establishment. Experience has shown that, if competent weed control is carried out, a density of 5–6 plants per square metre is adequate for these zones. Under good conditions this should result in 90% cover of desirable species within nine months of planting.

The Deep Marsh zone can be planted at four plants per square metre, and will take slightly longer to fill out (Fig. 2). Planting at a high density will allow desirable plants to quickly form an interlocking canopy that will help prevent erosion and weed invasion. The money saved from buying a smaller number of plants, and planting them sparsely, will soon be consumed by the cost of controlling weeds.

4. Species selection

When selecting which species to establish in a wetland, it is important to consider any information available on the physical, chemical and biological characteristics of the wetland environment. As mentioned above, factors such as wetland shape, soil types, salinity and pH will



Fig. 2. Restored near-coastal wetland at Torquay. This deep marsh area, planted at a density of four plants per square metre with Water Ribbon *Triglochin procerum* and Tall Spike-rush *Eleocharis sphacelata* has a good cover of aquatic species after 12 months of growth.

all influence which species will be most appropriate. Nearby intact remnant wetlands, with similar environmental characteristics to the area to be planted, are probably the most useful reference in determining what species to use for revegetation.

When selecting species, use some coloniser plants and begin a process of natural succession. Many wetland plants including species such as *Crassula helmsii*, *Elatine gratioloides*, *Lythrum hyssopifolium*, *Juncus bufonius* and *Isolepis*, other *Juncus* and *Epilobium* species fill the ecological niche of colonisers, and rapidly colonise bare areas that may otherwise be invaded by undesirable weeds. By purposefully planting a small proportion of coloniser species, it is possible to increase the speed at which desirable species reach a high cover and to put in place a soil seed bank which will protect the wetland in the event of any future disturbances.

5. Sourcing plant material

Using local genetic stock ensures that plants are well adapted to local soil and climatic conditions. It also decreases the likelihood of confu-

sion between species or subspecies for which the current taxonomy is unclear.

Aquatic and semi-aquatic plants can be grown in a range of formats. The format selected for each species should be determined by its growth form, size, habitat preferences and ecology. Large rhizomatous sedge species, including *Bamboo articulata* and *Eleocharis sphacelata*, establish best if planted from containers, such as 3 or even 6 inch pots, which allow the development of thick rhizomes and tall stems (over 1 metre in size). These characteristics maximise the success of establishment under conditions of high turbidity, wave action, avifaunal grazing and rapid water level fluctuations which can cause high plant mortality rates, particularly for plants planted in the shallow and deep marsh zones. Spreading herbaceous plants such as *Crassula helmsii* and *Myriophyllum* species are best grown in broad, shallow containers. Tussock-forming sedges and grasses, such as *Carex appressa* and *Poa labillardieri*, are best grown as tubestock or planting cells larger than 16 cm³. Using small-sized planting cells, less than 16 cm³, usually results in high plant mortality.

6. Planting and initial establishment

The timing of planting of aquatic and semi-aquatic species is crucial if successful establishment is to be maximised. The optimal time for planting is just before a species enters its optimal growing season. The growing season of wetland plants varies. Plants which grow on the fringe of wetlands often grow best in cool, moist conditions when there is plenty of moisture available in this zone, while true aquatics often grow most rapidly when the weather is warm.

Species which occur in deep water habitats are best planted when water levels are lowest in natural wetlands or before the normal high water level is reached in constructed wetlands. Once over the initial shock of planting, and if they are planted in their preferred growing season, these species are often quite capable of growing to keep up with a slow but steady increase in water level. Large rhizomatous sedge species such as *Baumea articulata* and *Eleocharis sphacelata*, should be planted at the shallow edge of their preferred habitat—i.e. in 200–300 mm of water—rather than out in deeper water, which is a hostile environment for establishing young

plants. Once well-established in the shallows, these plants will grow out into deeper water.

Plant Protection

A common cause of failure of establishment of some wetland species is that they are destroyed by grazing waterbirds while still small and vulnerable. If plants can be protected through the first growing season even the most palatable species can become established enough to survive the destructive feeding habits of birds such as Purple Swamp Hens or Black Swans. Protection of young plants is particularly crucial in degraded natural wetlands or constructed wetlands close to areas where there are established populations of wetland birds.

Protection is best achieved by suspending an enclosure made of netting or small gauge bird wire around young plants. This material should be arranged so that plants will have space to grow for a full growing season without becoming entangled within it, otherwise it becomes difficult to remove and re-use. Great care must be taken to ensure netting or wire is taut and that there are no points of entry into enclosed areas or wildlife may become entangled or trapped and drown.



Fig. 3. Given optimal conditions, including natural wetting and drying and protection from waterbird grazing, wetland vegetation can establish very rapidly. This sequence of photographs, taken over a nine month period, shows vegetation establishment in a constructed wetland from newly constructed and bare of native species on the above left to well vegetated with a high cover of indigenous plants and minimal weeds on the lower left.

7. Maintenance

In order to establish aquatic vegetation in wetlands, it is crucial to eradicate and suppress the establishment of certain key weeds by means of a well budgeted, designed and implemented weed control strategy, which includes timely application of appropriate methods, monitoring and follow up. High threat wetland weeds in Victoria are ranked in DSE (2008). Ideally weed control should be carried out by specialist contractors or skilled volunteers with proven ability to identify indigenous and weed species. If performed correctly, weed control will encourage regeneration of indigenous species and result in successful vegetation establishment.

8. Monitoring

Regular inspections of establishing wetland vegetation should be made over the first 12 months to monitor its progress and assess if adaptive management is required. The frequency of these inspections should be between every two to four weeks, and additional visits should be triggered by large rainfall events that may cause flooding. The checklist of things to

be monitored during each inspection include photo points (Fig. 3) plant health, weed invasion, the effects of rainfall and evaporation on water levels, any impacts from wave action, water salinity, turbidity and pH, impacts from pedestrian traffic or dog walking, wetland bird grazing and fauna colonisation.

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Bird vomit, the tour leader and the butcherbird 'sweet tooth'

While visiting Uluru last year, Fred Bohner, Kathy Himbeck and I decided to do the Mutitjulu Waterhole walk. While we were taking our time birdwatching and enjoying the scenery of this sacred place, a pair of Pied Butcherbirds *Cracticus nigrogularis* were seen regularly taking food back to their nest. After observing these birds for a few minutes we walked further and caught up to a tour group from a well-known Australian/New Zealand bus tour company. Not wanting to walk through the group, as their tour leader was talking with them, we stood back and listened to her spiel. Our interest in what she was saying was heightened when she started referring to a white coating all over the leaves of some small saplings as 'bird vomit'. On a closer inspection, after the group had moved on, the 'vomit' was found to be the

lerp casings of the nymph stages of psyllid insects. It was a heavy infestation on the sapling gums, as can be seen in Fig. 1.

Being a stickler for correcting misinformation, especially about natural history, I spoke to the guide quietly and let her know that the 'vomit' was in fact the sugary secretions of sap-sucking insects. She was going to check with her source of information, a local *Anangu* young man, who was a fellow tour leader. Whether the bird vomit relates to a traditional story, I have been unable to determine.

On our return walk from the waterhole, I observed an immature butcherbird on the ground, picking up items from the soil and eating them. As the bird was distracted by what it was doing, I was able to get close enough to take a series of photos. After the bird flew off, I walked over to

